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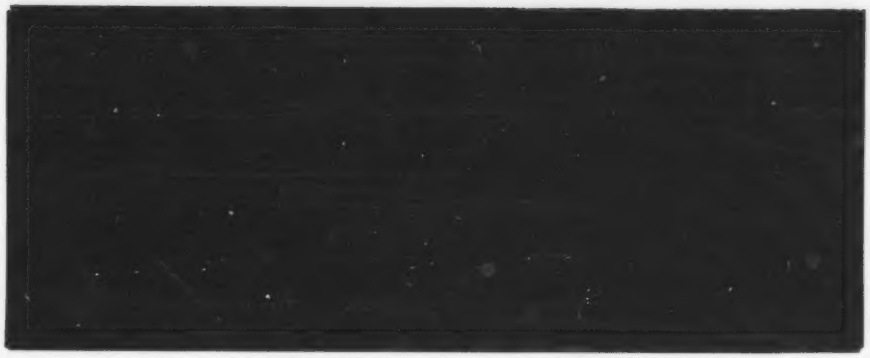

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TECHNICAL RESEARCH REPORT
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IMPROVE CANOLA MEAL FEED VALUE AND PRODUCE FOOD-GRADE PROTEIN BY ULTRAFILTRATION TECHNOLOGY

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1. EXECUTIVE SUMMARY

1.1 ABSTRACT

The efficiency of various extractants to simultaneously solubilize protein and antinutrients (phytate, sinapine, and glucosinolates) from commercial canola meal was investigated in a laboratory optimization experiment. One extractant (0.1 M EDTA, 0.4 M NaCl, pH 10.1) was selected for the simultaneous extraction of these components from canola meal on a pilot scale. About 20 percent of the protein and a majority of the antinutrients were extracted from the meal. Pilot scale ultrafiltration (UF) processing was applied to the meal extract to separate the protein from the antinutrients. Unfortunately, most of the antinutrients were concentrated with the protein under the conditions used. Alterations in extractant composition and/or UF membrane characteristics would probably result in a workable separation process.

1.2 SUMMARY

The overall objectives of this project were 1) to upgrade the feed value of canola meal by extracting the antinutrients (phytate, sinapine, and glucosinolates) and 2) to recover the simultaneously solubilized protein by separating the protein and antinutrients in the extract via ultrafiltration.

A literature review was conducted initially to identify solvents used by other investigators for solubilizing the protein, phytate, sinapine and glucosinolates in canola meal. Canola protein is most efficiently extracted at alkaline pH, although sodium chloride solutions and acid pH are also used. Ethylenediaminetetraacetic acid (EDTA) and sodium chloride (NaCl) were noted to be of value in solubilizing phytate. The solubility of phytate in relationship to pH is rather complex. Extractants containing methyl or ethyl alcohol are frequently used for sinapine and glucosinolates, but alcohols depress protein solubility.

A laboratory study was conducted utilizing response surface methodology to determine the optimum levels of pH, EDTA and NaCl for extracting the components of interest from canola meal. Twenty solvents containing various levels of these extraction aids were used to extract commercial canola meal (cv. Westar) at an extraction ratio of 1:8 (meal:solvent) for 30 minutes. After centrifugation of the slurry, the residual levels of protein, phytate, sinapic acid, and glucosinolates in the extracted meals were determined. It was noted that some component(s) of the extractant interfered with the assay for sinapine, thus sinapic acid content was measured instead. The various extractants solubilized 0 to 35 percent of the protein, 4 to 64 percent of the phytate, and appeared to solubilize 75 to 95 percent of the sinapic acid and glucosinolates. Mass balance analysis for sinapic acid and glucosinolates in the extracted meal and the extract from the pilot scale experiment indicated the solvent destroyed (rather than extracted) a majority of these components, as they were not accounted for in the two fractions. After statistical analysis of the laboratory data, an extractant composition (pH 10.1, 0.1 M EDTA, 0.4 M NaCl) was chosen for use in the pilot scale work. The choice of extractant composition was a compromise between protein and phytate solubility, as one extract did not optimally solubilize all components of interest.

Sixty-seven kg of canola meal was mixed with 509 kg of extractant in the pilot plant to produce 195 kg of extract under conditions analogous to those used in the laboratory. Portions of the extract were processed via ultrafiltration (UF) and diafiltration-ultrafiltration (DF-UF). These processes were expected to concentrate protein, which is retained by the membrane due to high molecular weight, and to eliminate antinutrients, which should pass through the membrane due to low molecular weight. The membrane used had an approximate 6000 molecular weight cutoff. The extract volume was reduced 4-fold (DF-UF) to 6-fold (UF) during 90 minutes of processing. This concentrated material was then spray-dried. The protein content of the spray-dried retentates (fraction retained by the membrane) was fairly low, 46 (UF) to 57 (DF-UF) percent. A high proportion of the ash (salt) content contributed by the extractant was not eliminated during the processing period resulting in retentate ash contents of 17 (DF-UF) percent and 25 (UF) percent. The higher protein and lower ash of the DF-UF retentate indicate that a longer diafiltration period would be necessary to reduce the ash and increase the protein to acceptable levels. A majority of the phytate, sinapic acid and glucosinolates present in the extract was also retained by the membrane, and thus was concentrated with the protein. This may have been due to poor permeability of the membrane to these compounds or to binding of these components to protein, creating molecules too large to pass through the membrane. Commercially available alternative membrane materials and molecular weight ranges should be investigated. In addition, use of an acidic extractant could reduce potential interactions between the canola proteins and antinutrients.

This project demonstrated 1) a feasible approach for investigating solvents for canola meal extraction, 2) the production of an improved canola meal, and 3) that commercial canola meal extracts can be processed via ultrafiltration without technical problems. Although a practical process was not identified, several useful processing options were recognized and deserve further investigation.

2. TECHNICAL REPORT

2.1 ABSTRACT

The possibilities of upgrading canola meal feed value by extracting antinutrients and of recovering the simultaneously-extracted canola protein for food use via ultrafiltration were investigated. First, the effects of varying extractant composition on the extractability of protein, phytate, sinapic acid and glucosinolates were studied via a laboratory, three factor response surface method experiment. The pH was varied between 8.5 and 10.5 and the concentrations of ethylenediaminetetraacetic acid (EDTA) and sodium chloride (NaCl) were varied between 0 and 0.5 M and 0 to 2.0 M, respectively, in the extractants. For the experimental points measured, these solvent combinations extracted 0 to 35 percent of the protein and 4 to 64 percent of the phytate. Meal content of sinapic acid and glucosinolates was reduced by 75 to 95 percent after extraction. but only small quantities were measured in the extractant. These compounds were probably chemically altered during extraction. No one extractant composition was optimum for solubilizing all the compounds measured, but an extractant composition of 0.1 M EDTA, 0.4 M NaCl and pH 10.1 was selected to prepare an extract from commercial meal for pilot scale ultrafiltration.

Ultrafiltration (UF) and diafiltration-ultrafiltration (DF-UF) processing were applied to the meal extract to separate the antinutrients (phytate, sinapic acid, and glucosinolates) from the protein and to concentrate the protein. The protein contents of the spray-dried retentates were fairly low, 46 percent (UF) and 57 percent (DF-UF), due to the high ash contents, 25 percent (UF) and 17 (DF-UF). The antinutrients present in the extract were concentrated with the protein during processing.

2.2 BACKGROUND AND OBJECTIVES

The use of canola meal as a high protein feed ingredient is limited to some extent by the presence of antinutrients, toxic constituents, and components contributing to poor sensory characteristics. These include fiber and phytate, low levels of glucosinolates, and sinapine. Food grade protein is not extracted from canola meal because of the difficulty in separating it from these compounds and poor recovery economics.

Ultrafiltration is a technique for separating dissolved or dispersed components on the basis of their molecular size. Within the food industry, ultrafiltration has been applied to the concentration of dairy proteins for 10 to 15 years and this technology is increasingly being applied to more diverse products. The low molecular weight and the solubility characteristics of the undesirable compounds in canola meal suggest that they may be removed from the meal by solubilization. The application of solvents to simultaneously extract undesirable components and solubilize a portion of the protein from meal could be followed by ultrafiltration of the solubles to separate extracted materials on the basis of molecular weight. Such a process would produce a detoxified canola meal and a food

grade protein material. Ultrafiltration studies of canola extract to remove phytate (Serraino and Thompson, 1984; Siy and Talbot, 1981, 1982) and glucosinolates (Diosady et al., 1984; von Bockelmann et al., 1977) have been investigated.

The objectives of this study were:

1. To identify solvents for simultaneous extraction of protein, phytate, sinapine and glucosinolates from commercially prepared canola meal, both by literature review and laboratory study, and

2. To separate the protein and low molecular weight undesirable compounds in the canola extract by pilot scale ultrafiltration processing.

The first step in this project was to review the literature to identify solvents used by other investigators to solubilize the protein and the low molecular weight, undesirable constituents (phytate, sinapine, and glucosinolates) of canola meal.

Protein. Much of the research on the solubility of canola/rapeseed proteins has utilized whole, unheated, seed or defatted seed meal prepared under low- or no-heat conditions. Eighty to 85 percent of the nitrogen in undenatured rapeseed meal can be extracted at pH 10 (30-45°C, 30 min, extraction ratio 1:20; Korolczuk and Rutkowski, 1971) and 67 percent of the nitrogen can be extracted by 10% sodium chloride (two extractions for 90 min, 5°C, extraction ratio 1:50; Bhatty et al., 1968). However, commercially available canola meal has undergone two heating steps during the course of processing. Heating a protein to above 50°C generally denatures it. In commercial processing of canola/rapeseed for oil extraction, the seed is cooked initially at 85°C for 30 min to inactivate myrosinase and to enhance oil removal. After solvent extraction of the screw-pressed cake, the meal is subjected to a toasting-desolventizing step at 100-130°C for 30 min to remove volatile glucosinolates (Finnigan and Lewis, 1985).

Several studies have quantitated the approximate one-third decrease in nitrogen solubility that occurs as a result of industrial heating of meal. A survey of rapeseed meals taken from a commercial processing plant over a three-day period revealed that an average of 43 percent of the nitrogen was soluble in 2.9% sodium chloride (extraction ratio 1:100, 1 h, 37°C; Seth and Clandinin, 1975). Ten percent sodium chloride extracts about 40 percent and 0.1N sodium hydroxide extracts 60 percent of the nitrogen in commercial meal (Girault, 1973). Insoluble nitrogen makes up about one-third of the nitrogen in rapeseed meals toasted at 110°C or less and relatively low humidity, but meals toasted at 120°C in a humid environment contain about 50% insoluble nitrogen (Rutkowski, 1970).

Finnigan and Lewis (1985) have recently investigated the optimization of protein extraction parameters for commercial rapeseed with the eventual aim of protein isolate production, possibly via ultrafiltration processing. About 40 percent of the meal nitrogen was extractable at pH 1.5 and 50

percent was extractable at pH 11.5 (extraction ratio 1:20, room temperature, 30 min). The use of a double extraction at pH 11 solubilized 57 percent of the nitrogen. At pH 11.5, the use of a 40 or 50°C extraction temperature increased nitrogen extractability as compared to 20 or 30°C. Other investigators have also utilized temperatures of 40 to 50°C to increase nitrogen solubility of commercial meals (Keshavarz et al., 1977; Girault, 1973). Use of sodium chloride or calcium chloride as the extractant (instead of water) improved nitrogen extractability over the pH range 2.5 to 9, but not at the pHs extremes (Finnigan and Lewis, 1985).

Other extractant additives may be useful in improving protein solubility. Jacks et al. (1983) have reported that exposure of protein isolates to 30% hydrogen peroxide markedly increased solubility, although Nakai et al. (1980) did not find a lov level of hydrogen peroxide (0.02%) improved the dispersibility of rapeseed protein isolate. Keshavarz et al. (1977) found hydrogen peroxide useful in improving the color of rapeseed protein extracted from commercially prep red meal. However, this strong oxidizing agent reduces protein nutritional value by causing oxidation of sulfur-containing amino acids (Keshavarz et al., 1977; Anderson et al., 1975). Nakai et al. (1980) found that sodium and potassium salts of myristic, oleic, and linoleic acids were effective for solubilizing rapeseed protein isolates prepared from commercial meal.

Phytate. Solubility of phytate is also largely dependent on the pH of the extracting solvent. pH is a major determinant of the nature and degree of interaction that occurs between phytic acid and protein in rapeseed. Phytic acid can bind to protonated basic residues of proteins at acidic pH, forming a binary protein-phytate complex. At alkaline pH, in the presence of cations, phytic acid forms a ternary protein-mineral-phytate complex (Serraino and Thompson, 1984). Phytate in a mildly-heated rapeseed sample was most soluble at pHs less than 1, around pH 4.5, and pH 10 (Gillberg and Törnell, 1976). Serraino and Thompson (1984) used dialysis (6000-8000 molecular weight cutoff) to remove phytate from 5 percent dispersions of rapeseed protein concentrate prepared from seed heated in 100°C water for 2 min. Dialysis at pH 3.5 removed 65 percent of the phytate. Addition of 0.25M ethylenediaminetetraacetic acid (EDTA) to the dispersion when dialysis was conducted at pH 9 removed 75 percent of the phytate. Siy and Talbot (1982) suggested that sodium chloride was the best dispersing agent for phytate extraction. They investigated sodium chloride concentration, extraction ratio, and pH to determine the optimum conditions for extracting phytate from commercial canola meal. Four percent sodium chloride (pH 6.3, extraction ratio 1:200) extracted 95 percent of the phytate. One percent sodium chloride, between pH 3 and 10, (extraction ratio 1:50) extracted about 70% of the phytate. At pHs below 3, a higher percentage of phytate was solubilized, and above pH 10, phytate extractability was variably related to pH. Rapeseed phytate is reported to have a minimum solubility at pH 11.7 (Gillberg and Törnell, 1976).

Solubilization of phytate from various materials in preparation for compositional assay is usually done with 1.2% or 2.4% hydrochloric acid (Haug and Lantzsch, 1983; Latta and Eskin, 1980), 0.5 M nitric acid (Davies

and Reid, 1979), 3% or 15% trichloroacetic acid (Knuckles et al., 1982; Uppström and Svensson, 1980), or 3% sulfuric acid (Camire and Clydesdale, 1982). Addition of EDTA during sample extraction improves the recovery of phytate (Ellis and Morris, 1983).

Sinapine. Sinapine content of Canadian rapeseed meals varies from 1.0 to 4.0% (Mueller et al., 1978; Blair and Reichert, 1984). Methods suggested for eliminating sinapine from rapeseed meal have included destruction by toasting or ammoniation and extraction with various solvents. Larsen et al. (1983) has suggested that toasting rapeseed meal decreases the sinapine content, but increases the content of sinapine derivatives, the effects of which are not well known. Under alkaline conditions sinapine is hydrolyzed to component sinapic acid and choline, and this reaction forms the basis for another method of reduction of sinapine content of rapeseed meal. Goh et al. (1982) found that steam sparging of canola meal in a desolventizer-toaster had little effect on sinapine content (contrary to Larsen et al., 1983), but sparging with steam plus ammonia (to create an alkaline environment) reduced the sinapine content of the meal by 65 percent. These authors also found that an ethanol extraction removed over 80 percent of the sinapine from Regent and Candle cultivars of commercially prepared canola meal, but only produced a 38 percent reduction for Tower meal. Dabrowski and Sosulski (1983) found extracting rapeseed flour (dehulled, defatted) with 70% ethanol or diffusion extraction of whole seeds with 0.01 N NaOH (80°C) to be more effective in removing esterified phenolic compounds than water extraction (80°C) of dehulled seeds or of dehulled, defatted flour.

Esterified phenolic acids (including sinapine) have been extracted from rapeseed materials in preparation for identification and/or quantitation by use of a mixture of methanol, acetone and water (7:7:6)(Krygier et al., 1982; Dabrowski and Sosulski, 1984), 80% aqueous methanol (Zadernowski et al., 1981; Mueller et al., 1978), 85% aqueous methanol (Kozlowska et al., 1975), 70% aqueous acetone (Fenton et al., 1980), and 60% acidified acetone (Ismail and Eskin, 1979). Zadernowski and Kozlowska (1983) tested several solvent systems, and found no significant differences in the amount and rate of extraction of phenolic compounds from highly defatted rapeseed flour with water, 80% methanol, 70% acetone, or a 1:1 mixture of 70% methanol and 70% acetone; however, they adopted 80% methanol as their choice of solvent. Dabrowski and Sosulski (1983) have suggested that no single solvent has been found effective to remove all phenolic constituents.

Glucosinolates. The removal of glucosinolates from rapeseed has been pursued rigorously in plant breeding programs. The 1985 average level of glucosinolates in Canadian canola was 25 μmoles/g, down from 80 μmoles/g in 1975 (Daun, 1986). Glucosinolate reduction is also achieved via processing. Forty to 60 percent of the glucosinolates present in the seed are destroyed during commercial processing via the desolventizer toaster (Daun, 1986). Extraction methods to remove glucosinolates have involved water and aqueous organic solvents. Rubin et al. (1984) has patented a method which utilizes 10% ammonia in methanol to extract 98% of seed

glucosinolates (Nazck et al., 1986). Sosulski and Dabrovski (1984) found a 70% ethanol extraction of canola flour to be more effective than vater or dilute alkaline solution for extracting glucosinolates. Van Megan (1983) noted that a 75% ethanol extraction of commercial rapeseed meal removed 99% of the glucosinolates. A myrosinase pretreatment to hydrolyze glucosinolates was found useful in their removal (van Megan, 1981). Ethanolic (50%) sodium hydroxide (0.01 N) has been used effectively to extract glucosinolates from whole rapeseed (Bhatty and Sosulski, 1972). Unfortunately, aqueous alcoholic solutions tend to denature and insolubilize protein.

Glucosinolates are highly soluble in water and several approaches to detoxifying canola/rapeseed protein materials are based on this. Woyevoda et al. (1978) used dilute alkali (pH 10) to simultaneously extract (triple extraction, ratio 1:6, 3 min) protein and glucosinolates from commercial rapeseed meal. Nearly all of the glucosinolates were extracted by this procedure. The glucosinolates were removed from the protein solution by use of an activated carbon treatment. Eapen et al. (1969) found that an aqueous extraction of commercial rapeseed meal (80°C, double extraction, ratio 1:30, 30 min) also almost completely removed glucosinolates and simultaneously solubilized 30 to 40 percent of the nitrogen.

Selection of Solvent. A wide variety of solvents have been employed for extracting the protein, phytate, sinapine and glucosinolates from canola/rapeseed meal. Since canola proteins are more soluble at alkaline pH, (Finnigan and Lewis, 1985), this range was chosen for study. In the alkaline pH range, phytate solubility is enhanced by addition of EDTA (Serraino and Thompson, 1984), so varying EDTA concentration in the extractant was tested. Sodium chloride is also reported to aid in phytate extraction (Siy and Talbot, 1982) and varying the concentration of NaCl was the third extractant parameter investigated. Sodium sulfite was added to all extractants because it has been reported to improve protein color (Jones and Holme, 1979; Blaicher et al., 1983; Keshavarz et al., 1977). Although alcohols have been reported useful for extracting sinapine and glucosinolates, their use was not considered because of their depressing effect on protein solubility. These components are also well extracted under alkaline conditions.

2.3 EXPERIMENTAL METHODS

One hundred kg of prepress, solvent-extracted canola meal (cv. Westar), processed under typical commercial conditions (including desolventizing-toasting), was obtained at POS Corp. This meal was used in laboratory and pilot plant experiments.

Laboratory. A three factor, rotatable design, response surface experiment (Mullen and Ennis, 1979; Ma and Ooraikul, 1986) was used to determine the optimum extractant level of pH, EDTA and NaCl for solubilizing the meal components of interest (protein, phytate, sinapine, and glucosinolates). A response surface method allows calculation of maximum extractabilities based on a few sets of experiments in which the

three solvent parameters are varied within chosen ranges simultaneously. It is more efficient than a one-factor-at-a-time approach (Ma and Ooraikul, 1986). The variables and the ranges tested were selected based on the literature review. The variable levels used are shown in Table 1. In rotatable designs employing 3 variables, 15 level combinations are tested and the center point is repeated 5 times. This enables a regression equation for the response surface to be calculated across the ranges tested for each response of interest (i.e. protein, phytate and sinapine and glucosinolate solubilization) (Mullen and Ennis, 1979). The level combinations used are shown along with the results (Table 3).

Table 1. Levels of variables used in the laboratory optimization experiments.

	Code				
	-1.682	-1	0	1	1.682
pH (X1)	8.5	8.9	9.5	10.1	10.5
EDTA concentration (X2)	0	0.10M	0.25M	0.40M	0.50M
NaCl concentration (X3)	0	0.4M	1.0M	1.6M	2.0M

The experiments were conducted by placing 21.02 g of canola meal (20.0 g of dry matter) into a 250 ml centrifuge bottle and adding 159 g of the appropriate extractant. The bottles were placed in a shaking water at 40°C for 30 min, maintaining a constant pH throughout the extraction. This was accomplished by monitoring pH every 5 min and adjusting as necessary with 4N NaOH. (The 4N NaOH also contained the appropriate concentrations of EDTA, NaCl and Na_2SO_3 to avoid dilution of these extract components during pH adjustment.) After extraction, the bottles were centrifuged for 3 min at 8,000 x g. The supernatant was decanted, weighed, and sampled for solids determination. The extracted meal residue was weighed, dried at 60°C in a vacuum oven, and assayed for protein, phytate, sinapine and glucosinolate content. These experiments were used to select the conditions for preparation of the canola meal extract for pilot scale ultrafiltration processing.

Pilot Plant. At the pilot scale, ultrafiltration and diafiltration of the canola meal extract was tested. Five hundred and nine kg of extractant containing 0.10M EDTA, 0.4M NaCl, and 1% (w/v) Na_2SO_3, adjusted to pH 10.1 with NaOH, was prepared in a 600 L portable tank. The extractant was heated to 40°C, then 66.7 kg of canola meal added. This slurry was stirred for 30 min, then passed over a 200 mesh vibratory screen (Model III

A-MS/MS, Rotex Inc., Cincinnati, OH) to recover the extracted meal solids and the canola extract. The extract was further clarified by passage through a 125 μm cartridge filter prior to ultrafiltration or diafiltration. The ultrafiltration (UF) unit, DDS LAB unit 37-165 (DDS, Nakskon, Denmark), had a membrane area of 1.65 m^2. The membranes were of a hydrophilic polymer cast directly on filter paper with a molecular weight cutoff of approximately 6000 (DDS type FS81PP). A preliminary pressure test of the UF unit was conducted in which flux response to increasing pressure was measured. This test indicated that the highest flux obtained before plateau was achieved at an average pressure of 5 mPa for the canola extract. This operating condition was used during UF or DF-UF processing of the extract. Processing was done in a batch mode. Both ultrafiltration and diafiltration coupled with ultrafiltration were conducted. The membranes were cleaned between processing runs by flushing with water, and then by recirculating a solution of 0.1% Alconox detergent and 0.2% household bleach, adjusted to pH 11 to 12 with sodium hydroxide, for 15 min at 40° to 50°C. The membranes were flushed with water again, and this cycle was repeated until a satisfactory flux rate was achieved.

Ultrafiltration. For UF processing, the feed tank was loaded with 50 kg of canola meal extract. The process was conducted at an average pressure of 5 mPa (6 mPa inlet pressure and 4 mPa outlet) and temperature of 40°C (39 to 42°C). Ultrafiltration was conducted for 90 min and the flux rate and samples of permeate and retentate taken throughout the run.

Diafiltration and Ultrafiltration. The DF-UF process was similar to UF processing except that for the first one-half of the process, one L of water (adjusted to pH 10.1) was added to the feed tank for every L of permeate output. This process kept the feed volume constant at 50 L to maintain the protein in a relatively dilute concentration to avoid reduction of flux rate (Cheryan, 1986). The DF process was continued for 35 L of permeate output, then UF processing carried out as previously described.

Spray-Drying. A portion of each retentate (recovered after processing) was neutralized to pH 7 to 8, then spray-dried in the laboratory (Büchi 190 Mini Spray-Drier, Büchi Laboratoriums-Technik AG, Switzerland) at 166°C inlet and 103°C outlet temperatures.

Analytical. Solids content of the supernatants was determined by drying approximately 9 g of material in a forced air oven at 105°C for 16 h. To avoid possible loss of heat labile components, the extracted meals were dried in a vacuum oven at 60°C for 16 h.

Nitrogen content was assayed using the automated Kjeldahl method (Kjel-Foss) (AOAC method 7.021, 1984). Nonprotein nitrogen (NPN) in the laboratory extracted meals and pilot plant samples was assayed to estimate the nitrogen contribution of the EDTA present in the extractant. One g of sample was stirred with 50 g of water (pH 10) for 30 min, then centrifuged 10 min at 10,000 x g. The supernatant was weighed and an equal weight of 20% (w/v) trichloroacetic acid added. After stirring for 15 min, this

material was centrifuged at 10,000 x g for 10 min. The supernatant was dried and assayed for nitrogen content. Percent NPN was subtracted from the Kjeldahl nitrogen to obtain protein nitrogen. Protein content was determined as nitrogen x 6.25.

Phytate content was determined by the method of Latta and Eskin (1980), and glucosinolates according to the Canadian Grain Commission method (Daun and McGregor, 1981). The contents of 3-butenyl, 4-pentenyl, 2-OH-3-butenyl, and 2-OH-4-butenyl glucosinolates were added together and are reported as total glucosinolates.

Sinapine (Reinecke salt precipitation) and sinapic acid were determined according to Mueller et al. (1978). However, during the analysis it appeared that one or more of the extractant components (NaOH, EDTA, NaCl or Na_2SO_3) interfered with the Reinecke salt determination for sinapine. This was verified by spiking unextracted canola meal with extractant components at a level approximating that estimated to be residual in the extracted meal. Optical density values for sinapine obtained for the spiked meal were depressed to equal those of the blank. By omitting the Reinecke salt precipitation, this method can be used to measure sinapic acid (hydrolysed from all sinapic acid esters, not just sinapine). The determination of sinapic acid in extracted and unextracted-spiked meals did not seem to be affected by the presence of extractant components. However, during pilot scale processing, the slurry of canola meal-extractant contained only 7 percent of the sinapic acid present in the original meal. Whether this was due to alteration or to binding of the sinapic acid, rendering it unmeasurable by this method, or due to interference of the extractant components in the assay, was not investigated. For all laboratory extracted meals and pilot plant samples, the sinapic acid content is reported. However, the values obtained approached the detection limits of the method and thus must be used with caution. Hydrolysed sinapine bisulfate was used to prepare the standard curve.

2.4 RESULTS AND DISCUSSION

Laboratory. The composition of the Westar canola meal (starting material for extraction) was 4.9% moisture, and on a dry matter basis: 39.6% protein, 3.73% phytate, 2.3% sinapine, and 7.0 μmoles/g glucosinolates. The data obtained from the rotatable design experiments on varying the extractant composition and its effect on the solubility of the protein, phytate, sinapic acid and glucosinolates in the meal are shown in Table 2 for the level combinations tested. These components were measured in the extracted meal, since this was more convenient than analyzing the extracts. The data are presented as the amount of component present in the extracted meal as a percentage of that in the starting material. Assuming no destruction or binding of the components occurred during extraction, subtraction of these values from 100 would provide the percentage of the component extracted into the supernatant. (Analysis of the extraction slurry, the extracted meal, and the extract (supernatant) in the pilot scale experiments to be discussed later, indicated that this assumption would not be valid for sinapine and glucosinolates.)

Table 2. Protein, phytate, sinapine and glucosinolates yields recovered in extracted meal as a percentage of that originally present in unprocessed meal (starting material).

Extractant Composition[a]			Percentage Remaining in Meal			
pH	EDTA	NaCl	Protein	Phytate	Sinapic Acid	Total Glucosinolates
8.9	0.10M	0.4M	78.6	52.9	12	25
8.9	0.10M	1.6M	73.6	60.9	10	27
8.9	0.40M	0.4M	90.4	43.8	5	4
8.9	0.40M	1.6M	83.7	74.9	9	21
10.1	0.10M	0.4M	72.7	38.0	21	21
10.1	0.10M	1.6M	77.4	41.0	17	14
10.1	0.40M	0.4M	98.0	66.6	25	13
10.1	0.40M	1.6M	87.7	41.1	24	19
10.5	0.25M	1.0M	85.9	42.5	20	12
8.5	0.25M	1.0M	85.1	44.9	14	20
9.5	0.50M	1.0M	99.9	63.9	24	20
9.5	0	1.0M	65.1	95.6	11	20
9.5	0.25M	2.0M	79.3	36.2	22	15
9.5	0.25M	0	81.2	40.4	15	23
9.5	0.25M	1.0M	85.4	35.8	14	18
9.5	0.25M	1.0M	81.8	37.9	16	19
9.5	0.25M	1.0M	82.5	38.6	19	16
9.5	0.25M	1.0M	84.4	33.8	16	17
9.5	0.25M	1.0M	80.1	37.6	22	20
9.5	0.25M	1.0M	86.3	36.3	19	20

[a] All extractants contained 1% (w/v) Na_2SO_3.

The data in Table 2 were statistically analysed using the General Linear Model of SAS (SAS Institute, Inc. 1985) to fit the regression equation. In order to visualize the equation, response surfaces (contour plots) of EDTA concentration vs. NaCl concentration were plotted at five pH levels (8.5, 8.9, 9.5, 10.1, 10.5) for each of the components measured (protein, phytate, sinapine and glucosinolates). The model equations are presented in Table 3 along with the R-square and the probability, which are measures of fit between the model and the data. The models for sinapic acid and glucosinolates were not significant at the 5% level of probability.

Table 3. Response surface models for protein, phytate, sinapic acid and glucosinolates, and their significance.

Component	Model[a]	Significance	R-Square
Protein	$y = 306 - 45.9X1 - 93.8X2 - 10.6X3 + 2.1X1^2 - 14.0X2^2 - 3.1X3^2 + 19.0X1X2 + 2.1X1X3 - 23.2X2X3$	***	0.92
Phytate	$y = 471 - 89.8X1 - 64.7X2 + 20.6X3 + 5.1X1^2 + 66.2X2^2 - 0.06X3^2 + 33.1X1X2 - 21.4X1X3 - 7.5X2X3$	*	0.78
Sinapic Acid	$y = -699 + 151X1 - 252X2 + 18.9X3 - 7.9X1^2 + 1.3X2^2 + 1.1X3^2 + 26.4X1X2 - 2.4X1X3 + 12.5X2X3$	NS	0.47
Glucosinolates	$y = 110 - 14.0X1 - 375X2 + 57.6X3 + 0.6X1^2 + 9.5X2^2 - 0.4X3^2 + 33.3X1X2 - 6.9X1X3 + 35.9X2X3$	NS	0.67

[a] y = response (percentage of protein, phytate, sinapic acid or glucosinolates); X1 = pH (8.5 to 10.5); X2 = molarity of EDTA (0 to 0.50); X3 = molarity of NaCl (0 to 2.0).
NS = Not significant
* Indicates significance at 5% level.
*** Indicates significance at 0.1% level.

Protein. Extraction solubilized between 0 and 35 percent of the protein present in the meal for the level combinations measured (Table 2). Examination of the response surface contour plots predicted by the model indicated that increasing the pH from 8.5 to 10.5 would increase the extractability of the protein. An increase from about 30 percent of total protein extracted at pH 8.5 to about 40 percent at pH 10.5 was predicted at optimum NaCl and EDTA concentrations. The effects of extractant NaCl concentration on protein extractability varied slightly with the pH level. As pH increased, increasing salt concentration depressed protein extraction. The effect of increasing extractant EDTA concentration was to decrease protein extractability sharply. However, as NaCl concentration was increased, the depression of protein solubility due to EDTA was somewhat mediated.

Phytate. At the level combinations of extractant tested, between 4 and 64 percent of the canola meal phytate was extracted. Examination of the response surface contour plot for phytate revealed that a concentration of 0.24 to 0.27 M EDTA uniformly extracted the most phytate across all pH and NaCl levels tested, with higher or lower concentrations of EDTA resulting in lower phytate extractions. Phytate extraction was interactively affected by the pH level and the NaCl concentration of the extractant, but the effects were small. At pH 9.5 and below, higher quantities of phytate were extracted with the lowest concentrations of NaCl present in the extractant. At pHs 10.0 to 10.5, phytate was extracted slightly better at the higher salt concentrations (up to 2.0 M).

Sinapic Acid. According to Table 2, between 75 and 95 percent of the sinapic acid present in the meal was extracted using the level combinations of extractant shown. However, the validity of the sinapic acid assay is in doubt, as discussed earlier, and thus these values must be used with caution. The regression equation for the effects of extractant components on the extractability of sinapic acid fitted poorly with the observed data (Table 3). However, the general trends observed in the contour plots will be reported. The extractability of sinapic acid appeared to be affected by pH, EDTA and NaCl levels interactively. At pH values below 9.0, high EDTA and low NaCl concentrations extracted more sinapic acid than other level combinations, but at pH values above 9.5, low EDTA and high NaCl appeared to extract slightly more sinapic acid. High concentrations of both salt and EDTA across all pHs uniformly extracted the least sinapic acid.

Glucosinolates. As will be seen during discussion of the pilot plant data, the extractants used probably destroyed a large percentage of the glucosinolates present. The glucosinolate content of the meals was near the quantitation limits for the assay and the differences among the various extractant treatments were small. The extracted meals contained from 0.7 to 1.5 μmoles glucosinolates/g (Table 2). The fit of the model equation to the observed data for the extraction of glucosinolates was poor (Table 3). Glucosinolate solubility responded to changes in pH, EDTA, and NaCl levels in the extractant in a manner analogous to that of sinapic acid extraction, except that the extractants with the lowest levels of EDTA and NaCl extracted the least glucosinolates, across all pH levels.

Selection of Extractant. From the preceding discussion, it is apparent that altering the extractant composition affected the extraction of the components of interest in different ways. Increasing pH from 8.5 to 10.5 improved the extractability of protein. This pH range generally caused a decrease, followed by an increase at around pH 10.0, in the extractability of phytate, sinapic acid and glucosinolates. Increasing the EDTA concentration of the extractant depressed protein extractability. At pHs above 10, increasing EDTA concentration also depressed sinapic acid and glucosinolate extractabilities. (At pHs below 10, higher levels of EDTA seemed to be beneficial). However, a level of about 0.25 M EDTA appeared to be optimum for phytate solubilization. Increasing the concentration of NaCl in the extractant had a neutral or slightly negative effect on protein extraction. Increased NaCl concentration in the extractant decreased

phytate extraction at the lover pHs, but enhanced it at higher pHs studied. For sinapine and glucosinolate extraction, the effects of increasing NaCl concentration were dependent on pH and EDTA concentration, but in general at the higher pHs, a higher concentration of NaCl was desirable for increased extraction. Thus, the selection of an extractant for the pilot scale process was not straight forvard. Based on examination of the contour plots, a pH of 10.1 was selected. Selection of a optimum level of EDTA was not possible, but a concentration of 0.1M was selected to improve the extractability of phytate, although EDTA would depress extractability of some other compounds of interest. A salt concentration of 0.4 M NaCl was selected, although high NaCl levels (to 2.0 M) appeared to improve sinapic acid and glucosinolate extractability. This level was kept low to avoid a very high solids contribution of the extractant.

Pilot Plant. The compositions of the starting material and the products of pilot scale processing are shown in Table 4.

Table 4. Composition (dry matter basis) of canola meal, pilot plant extracted meal, and spray-dried ultrafiltered (UF) and diafiltered-ultrafiltered (DF-UF) proteins.

Starting material	Protein	Phytate	Sinapic Acid	Glucosinolates
	%			µmoles/g
Canola meal	39.6	3.73	1.55	7.0
Recovered fractions				
Extracted meal	25.7	1.15	0.07	0.3
UF protein	45.7	5.19	0.26	1.3
DF-UF protein	56.9	4.65	0.23	1.0

Extraction. Pilot plant extraction of canola meal solubilized 20 percent of the protein and 50 percent of the phytate. This is somewhat lover than that predicted by the laboratory experiments of 27 percent protein and 62 percent phytate. As mentioned previously, the quantitation of sinapic acid presented some difficulty. The canola meal extract contained only 2 percent and the extracted meal contained 5 percent of the sinapic acid present in the original canola meal. The analogous laboratory-extracted meal contained 20 percent of the original sinapic acid. Similarly the glucosinolate content of the meal was nearly destroyed under the extraction conditions used. No glucosinolates were measured in the extract before concentration, and the extracted meal contained 5 percent of that originally present in the unextracted meal.

Processing. Ultrafiltration or ultrafiltration-diafiltration processing of the meal extract was successful in concentrating protein and reducing ash content, but not in removing phytate, sinapic acid or glucosinolates. The ultrafiltration or diafiltration-ultrafiltration processing of the extract vent smoothly. The change in flux rate over time for the two processes is shown in Fig. 1 and the changes in the fractions (retentate and permeate) weight and solids distribution are shown in Figs. 2 and 3. After 90 min, the flux rate had been reduced from about 0.9 kg/min to 0.2 (UF) to 0.4 (DF-UF) kg/min. As would be expected, a high flux rate was maintained during diafiltration (Fig. 1). The feed weight (retentate) was reduced from 50 kg to 8 kg (UF) or 14 kg (DF-UF) after 90 min of processing, a 4- to 6-fold decrease. At this point, the retentate had become quite viscous. Thirty (UF) to 39 (DF-UF) percent of the original feed solids were found in the retentate after processing. Because of the high quantities of EDTA, NaCl, NaOH and Na_2SO_3 in the extractant solution, 40 percent of the dry solids weight in the extraction slurry was contributed by the extractant and 60 percent was contributed by the canola meal. Thus, much of the solids reduction during UF or DF-UF processing was due to the loss of extractant solute.

Protein. During processing, 85 to 88 percent of the protein was retained by the membrane. The remainder, low molecular weight material, was lost in the permeate. Considering that 20 percent of meal protein was extracted, this process recovered 18 percent of the protein present in the canola meal starting material. The protein content of the spray-dried retentates was low, 46 (UF) to 57 (DF-UF) percent, and the ash content unacceptably high, 17 (DF-UF) to 25% (UF). The improved protein and ash contents of the DF-UF protein material, as compared to the UF process, suggests a longer period of diafiltration is required to reduce the ash to acceptable levels. An ash content of 4 to 5 percent is considered acceptable in plant-derived protein food products.

Phytate. Only about 50 percent of the phytate present in the canola meal extract was recovered in the rententates and permeates of both processes. The unaccounted for phytate may have complexed with proteins during processing so that it was not measured during the phytate assay. The measured phytate partitioned primarily into the retentate (90%), contrary to expectations. This was probably due to formation of a protein-mineral-phytate complex (Serraino and Thompson, 1984) which was retained by the membrane. The phytate content of the spray-dried protein materials (Table 4) was about two times that found in soy protein isolates (Brooks and Morr, 1985).

Sinapic Acid and Glucosinolates. Due to the disappearance of approximately 95 percent of the sinapic acid and glucosinolates during the canola meal extraction, the quantities for analysis in the ultrafiltered retentates and permeates were very low. About 60 percent of the measured sinapic acid was retained by the membrane, as was nearly all the measurable glucosinolates. This was contrary to expectations and suggests that either this membrane was not permeable to sinapic acid and glucosinolates or that the extractant solution promoted binding of these materials, creating

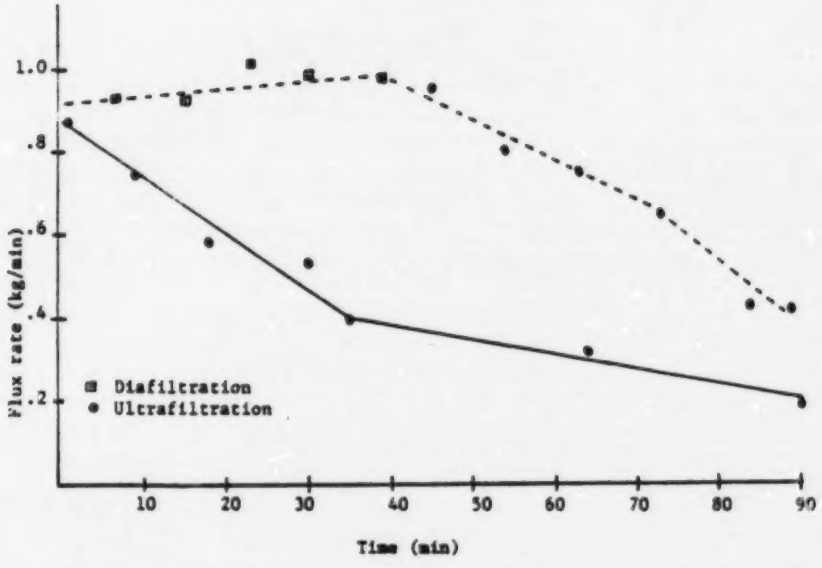

Figure 1. Flux rate over time during ultrafiltration and diafiltration-ultrafiltration processing of canola meal extract.

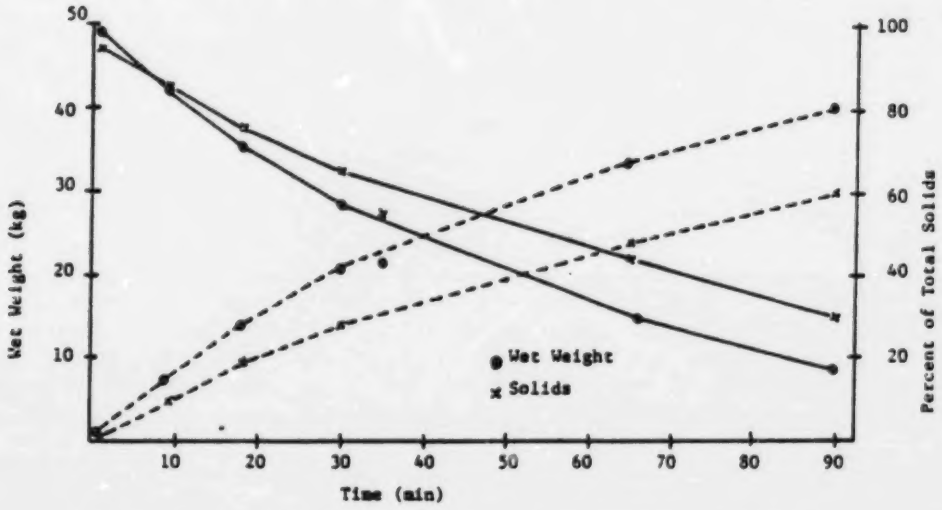

Figure 2. Ultrafiltration retentate (——) and permeate(---) cumulative wet weight and solids content during processing.

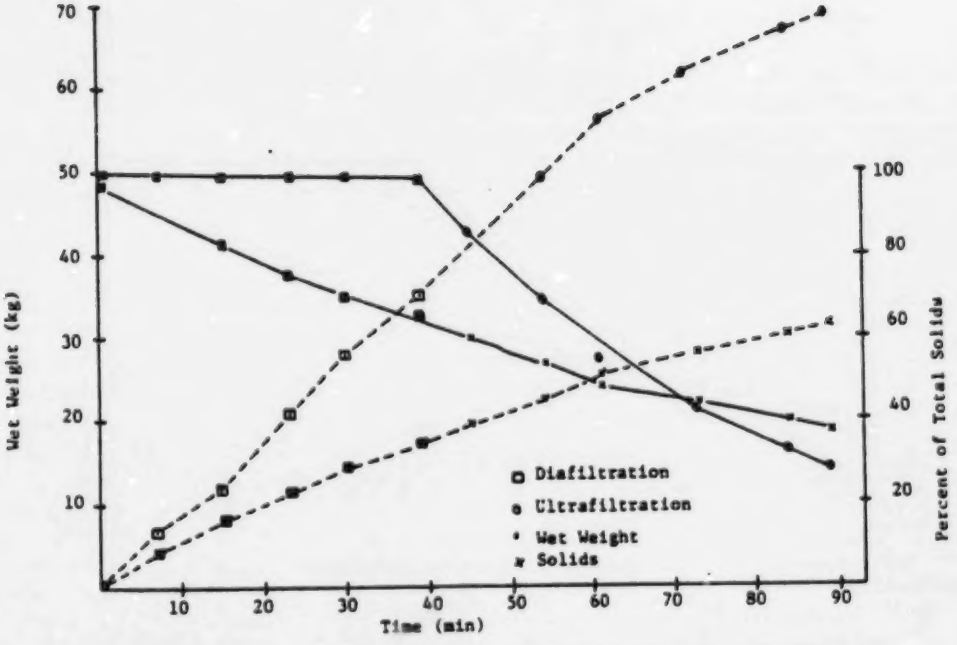

Figure 3. Diafiltration-ultrafiltration retentate(——) and permeate (---) cumulative wet weight and solids content during processing.

molecules too large to pass through the membrane. The sinapic acid and glucosinolate contents of the spray-dried protein materials, however, was considerably less than that of the starting material (Table 4).

2.5 CONCLUSIONS AND IMPORTANCE

The extractant combinations for which data were collected in the laboratory phase of this project solubilized up to 35 percent of the protein and up to 64 percent of the phytate from commercial canola meal. Sinapic acid and glucosinolates were probably destroyed to a large extent by the extraction procedure. Response surface methodology models for protein and phytate predicted different extractant compositions for optimum solubilization of these components. Predictive models for sinapic acid and glucosinolate extraction in response to extractant composition did not fit the data well. However, use of the rotatable design, response surface method to optimize solvent composition was an efficient technique for achieving project objectives.

The extraction of commercial canola meal to produce a detoxified meal was successful. The levels of phytate, sinapic acid and glucosinolates in the extracted meal were substantially reduced as compared to the starting material (Table 4).

Ultrafiltration processing of the canola meal extract concentrated the protein and was operated without technical problems. However, the phytate, sinapic acid and glucosinolates present in the extract were mostly retained by the membrane, in spite of their low molecular weights. The extractant composition and the concentration process may have promoted binding of these components with protein. An alternate membrane composition and/or molecular weight cutoff might improve permeation of the low molecular weight components and changes in extractant composition could reduce binding. A longer period of diafiltration processing should be used to decrease the ash level in the spray-dried retentate.

The application of extraction and ultrafiltration processing to upgrade canola meal turned out to be more complex than envisioned in the project proposal. The procedures investigated in this project cannot be recommended for commercial use at this time. However, the procedure worked well enough to suggest that further investigation of potential extractants and alternative membranes could result in a feasible processs. The extractant variables examined in the project were limited to three. Further study to test other, promising extractant options identified by the literature review is recommended, utilizing the methodology developed in the project. This includes the use of low pH to improve phytate solubility and to decrease protein-antinutrient binding and the investigation of surfactants as solubilizers. The use of meal obtained prior to the desolventizer-toaster to improve component solubility should also be investigated. The opportunity to improve canola meal utilization by producing detoxified meal and food grade proteins varrants further investigation.

2.6 REFERENCES

Anderson, G.H., Li, G.S.K., Jones, J.D., and Bender, F. 1975. Effect of hydrogen peroxide treatment on the nutritional quality of rapeseed flour fed to weanling rats. J. Nutr. 105:317.

AOAC. 1984. "Official Methods of Analysis of the Association of Official Analytical Chemists". 14th Edition, AOAC, Washington, D.C.

Bhatty, R.S. and Sosulski, F.W. 1982. Diffusion extraction of rapeseed glucosinolates with ethanolic sodium hydroxide. J. Amer. Oil Chem. Soc. 49:346.

Bhatty, R.S., McKenzie, S.L., and Finlayson, A.J. 1968. The proteins of rapeseed (Brassica napus L.) soluble in salt solutions. Can. J. Biochem. 46:1191.

Blaicher, F.M., Elstner, F., Stein, W., and Mukherjee, K.D. 1983. Rapeseed protein isolates: Effect on processing on yield and composition of protein. J. Agric. Food Chem. 31:358.

Blair, R. and Reichert, R.D. 1984. Carbohydrate and phenolic constituents in a comprehensive range of rapeseed and canola fractions: nutritional significance for animals. J. Sci. Food Agric. 35:29.

Brooks, J.R. and Morr, C.V. 1985. Effect of phytate removal treatmments upon the molecular weight and subunit composition of major soy protein fractions. J. Agric. Food Chem. 33:1128.

Camire, A.L. and Clydesdale, F.M. 1982. Analysis of phytic acid in foods by HPLC. J. Food Sci. 47:575.

Cheryan, M. 1986. Ultrafiltration Handbook. Technomic Publ. Co., Inc., Lancaster, PA.

Dabrowski, K. and Sosulski, F. 1983. Extraction of phenolic compounds from canola during protein concentration and isolation. In: Proceedings 6th International Rapeseed Conf. Vol. II. Groupe Consultatif International de Recherche sur le Colza, Paris, May 18-19, p. 889.

Dabrowski, K. and Sosulski, F. 1984. Quantitation of free and hydrolyzable phenolic acids in seeds by capillary gas-liquid chromatography. J. Agric. Food Chem. 32:123.

Daun, J.K. 1986. Glucosinolate levels in Western Canadian rapeseed and canola. J. Amer. Oil Chem. Soc. 63:639.

Daun, J.K. and McGregor, D.I. 1981. Glucosinolate analysis of rapeseed (canola). Method of the Canadian Grain Commission, Grain Research Laboratory, Agric. Canada, Winnipeg, MB.

Davies, N.T. and Reid, H. 1979. An evaluation of the phytate, zinc, copper, iron and manganese content of, and Zn availability from soya-based textured-vegetable-protein meat substitutes or meat-extenders. Br. J. Nutr. 41:579.

Diosady, L.L., Tzeng, Y.-M., Rubin, L.J. 1984. Preparation of rapeseed protein concentratess and isolates during ultrafiltration. J. Food Sci. 49:768.

Eapen, K.E., Tape, N.W. and Sims, R.P.A. 1969. New process for the production of better quality rapeseed oil and meal. II. Detoxification and dehulling of rapeseeds-feasibility study. J. Amer. Oil Chem. Soc. 46:52.

Ellis, R. and Morris, E.R. 1983. Improved ion-exchange phytate method. Cereal Chem. 60:121.

Fenton, T.W., Leung, J., and Clandinin, D.R. 1980. Phenolic components of rapeseed meal. J. Food Sci. 45:1702.

Finnigan, T.J.A. and Lewis, M.J. 1985. Nitrogen extraction from defatted rapeseed, with particular reference to United Kingdom commercial rapeseed meal. J. Sci. Food Agric. 36:520.

Gillberg, L. and Törnell, B. 1976. Preparation of rapeseed protein isolates. Dissolution and precipitation behavior of rapeseed proteins. J. Food Sci. 41:1063.

Girault, A. 1973. The study of some properties of rapeseed protein with a view to protein concentrate production. J. Sci. Food Agric. 24:509.

Goh, Y.K., Shires, A., Robblee, A.R. and Clandinin, D.R. 1982. Effect of ammoniation of rapeseed meal on the sinapine content of the meal. Poul. Sci. 23:121.

Haug, W. and Lantzsch, H.-J. 1983. Sensitive method for the rapid determination of phytate in cereals and cereal products. J. Sci. Food Agric. 34:1423.

Ismail, F. and Eskin, N.A.M. 1979. A new quantitative procedure for determination of sinapine. J. Agric. Food Chem. 27:917.

Jacks, T.J., Hensarling, T.P. and Muller, L.L. 1983. Increased oilseed protein solubility after peroxide exposure. J. Amer. Oil Chem. Soc. 60:852.

Jones, J.D. and Holme, J. 1979. Oilseed processing. U.S. patent 4,158,656.

Keshavarz, E., Cheung, R.K.M., Lui, R.C.M. and Nakai, S. 1977. Adaptation of the three stage extraction process to rapeseed meal for preparation of colorless protein extracts. Can. Inst. Food Sci. Technol. J. 10:73.

Knuckles, B.E., Kuzmicky, D.D., and Betschart, A.A. 1982. HPLC analysis of phytic acid in selected foods and biological samples. J. Food Sci. 47:1257.

Korolczuk, J. and Rutkovski, A. 1971. Extraction of N-compounds of rapeseed meal in relation to pH and temperature. J. Amer. Oil Chem. Soc. 48:398.

Kozlovska, H., Sabir, M.A., Sosulski, F.W. and Coxworth, E. 1975. Phenolic constituents in rapeseed flour. Can. Inst. Food Sci. Technol. J. 8:160.

Krygier, K., Sosulski, F. and Hogge, L. 1982. Free, esterified, and insoluble-bound phenolic acids. 1. Extraction and purification procedure. J. Agric. Food Chem. 30:330.

Larsen, L.M., Olsen, O., Plöger, A., and Sørensen, H. 1983. Phenolic choline esters in rapeseed: possible factors affecting nutritive value and quality of rapeseed meal. In: Proceedings 6th Internatl. Rapeseed Conf. Vol II. Groupe Consultatif. International de Recherche sur le Colza, Paris, 17-19 May, p. 1577.

Latta, M. and Eskin, M. 1980. A simple and rapid colorimetric method for phytate determination. Agric. Food Chem. 28:1313.

Ma, A.Y.M. and Ooraikul, B. 1986. Optimization of enzymatic hydrolysis of canola meal with response surface methodology. J. Food Proc. Preserv. 10:99.

Mullen, K. and Ennis, D.M. 1979. Rotatable designs in product development. Food Technol. 33(7):74.

Mueller, M.M., Ryl, E.B., Fenton, T., and Clandinin, D.R. 1978. Cultivar and growing location differences on the sinapine content of rapeseed. Can. J. Anim. Sci. 58:579.

Nakai, S., Ho, L., Tung, M.A., and Quinn, J.R. 1980. Solubilization of rapeseed, soy and sunflower protein isolates by surfactant and proteinase treatment. Can. Inst. Food Sci. Technol. J. 13:14.

Nazck, M., Shahidi, F., Diosady, L.L., and Rubin, L.J. 1986. Removal of glucosinolates from Midas rapeseed and mustard seed by methanol-ammonia. Can. Inst. Food Sci. Technol. J. 19:75.

Rubin, L.J., Diosady, L.L. and Phillips, C.R. 1984. Solvent extraction of oil bearing seeds. U.S. patent 4,460,504.

Rutkovski, A. 1970. Effect of processing on the chemical composition of rapeseed meal. In: Proc. Internatl. Conf. Science, Technology and Marketing of Rapeseed Products, St. Adele, PG, Publ: Rapeseed Association of Canada in cooperation with Dept. of Industry, Trade and Commerce, Ottawa.

SAS Institute, Inc. 1985. SAS User's Guide: Statistics, Version 5 Edition, Cary, N.C.

Serraino, M.R. and Thompson, L.U. 1984. Removal of phytic acid and protein-phytate acid interactions in rapeseed. J. Agric. Food Chem. 32:38.

Seth, P.C.C. and Clandinin, D.R. 1975. Variation in the solubility of nitrogen in prepress-solvent processed rapeseed meal. Poul. Sci. 54:297.

Siy, R.D. and Talbot, F.D.F. 1982. Preparation of lov-phytate rapeseed protein by ultrafiltration. I. The aqueous extraction of phytate from deoiled rapeseed meals. J. Amer. Oil Chem. Soc. 59:191.

Siy, R.D. and Talbot, F.D.F. 1981. Preparation of lov-phytate rapeseed protein by ultrafiltration. II. Membrance development and testing. J. Amer. Oil Chem. Soc. 58:1021.

Sosulski, F.W. and Dabrowski, K.J. 1984. Determination of glucosinolates in canola meal and protein products by desulfation and capillary gas-liquid chromatography. J. Agric. Food Chem. 32:1172.

Uppström, B. and Svensson, R. 1980. Determination of phytic acid in rapeseed meal. J. Sci. Food Agric. 31:651.

van Megan, W.H. 1983. Removal of glucosinolates from defatted rapeseed meal by extraction with aqueous ethanol. Can. Inst. Food Sci. Technol. J. 16:93.

van Megan, W.H. 1981. Process for producing a detoxified rapeseed concentrate. U.S. patent 4,244,973.

von Bockelmann, I., Dejmek, P., Ericksson, G. and Hallström, B. 1977. "Potential applications in food processing." In: Reverse Osmosis and Synthetic Membranes, Souriragan, S., Ed., National Res. Council, Ottava, Pub. No. NRCC 15627, p. 445.

Voyevoda, A.D., Nakai, S. and Watson, E.L. 1978. Detoxifiction of rapeseed protein products by an activated carbon treatment. Can. Inst. Food Sci. Technol. J. 11:107.

Zadernovski, R. and Kozlovska, H. 1983. Phenolic acids in soybean and rapeseed flours. Lebensm.-Wiss. u. Technol. 16:110.

Zadernovski, R., Rotkievicz, D., Kozlovska, H., Sosulski, F. 1981. The procedure of determination different form of phenolic acid in rapeseed flours. Acta Aliment. Polon. 7:147.

3. EXPENDITURES AND PERSONNEL

Manpower	Units (hr)	Dollars
Senior Scientists		
Sandra McCurdy	229.5	$10,040.69
Paul Fedec	1.5	65.63
Scientist		
Myles Marianchuk	13.5	492.75
Process Engineers		
Dennis Trumpy	21.0	567.00
Gregg Willie	6.0	219.00
Tradesmen		
Lloyd Frank	4.0	146.00
Dixon Cox	12.5	456.25
Technicians		
Catherine Greuel	267.5	6,152.50
Jan Ellenberger	55.5	1,276.50
Librarian		
Mark Reineke	3.0	109.50
Operators	118.0	2,714.00
Support Staff	41.0	686.54
	TOTAL MANPOWER	$22,926.36

Equipment Rental	
POS Equipment	1,715.63
DDS Ultrafiltration Unit Rental (including shipping)	5,111.11
TOTAL EQUIPMENT RENTAL	$6,826.74

Supplies	
Library charges (reprints and computer search)	50.86
Chemicals	1,633.66
UF Hollow Fiber Membrane	800.00
TOTAL SUPPLIES	$2,484.52
TOTAL EXPENDITURES	$32,237.62

All POS Corp. manpower and equipment were charged at member rates (a 25% discount).